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# THE HARD ROT DISEASE OF GLADIOLUS

A THESIS

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL  
OF CORNELL UNIVERSITY FOR THE DEGREE OF  
DOCTOR OF PHILOSOPHY

BY

LOUIS MELVILLE MASSEY

Published as Cornell University Agricultural Experiment Station Bulletin 380,  
September 1916





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# THE HARD ROT DISEASE OF GLADIOLUS<sup>1</sup>

L. M. MASSEY

## THE HOST PLANT

The gladiolus is a cormous, summer-flowering plant. Pax (1889)<sup>2</sup> classifies it as a member of the family Iridaceae, of the tribe Ixiodeae, subtribe Gladioleae, genus Gladiolus. The species of Gladiolus may be grown from corms, from cormels (the grayish to black, hard-shelled bodies formed on underground stems at the base of the new corm), or from seed. The plants are indigenous to South Africa, where, according to Crawford (Crawford and Van Fleet, 1911:3), about fifty species have been discovered. This writer states:

It is also a native of middle Africa, central and southern Europe, Persia, Caucasus, and the country around the eastern end of the Mediterranean. About forty additional species have been found in these localities, and one in Hampshire, England. These have been hybridized and crossed until they are so mixed that it is impossible for the ordinary grower to say what blood may have entered a given variety—nor does it matter.

## ECONOMIC IMPORTANCE OF THE GLADIOLUS INDUSTRY

According to Hendrickson (1911), there are from four hundred to five hundred acres in the United States devoted to gladioli, the annual production of corms being from 14,000,000 to 15,000,000 and the estimated value of the crops \$250,000. In New York State, besides many small growers there are two growers each having over one hundred acres devoted entirely to the cultivation of gladioli. A list of the members of the American Gladiolus Society which appeared in 1914 in the *Modern Gladiolus Grower* (1:31-32) contains two hundred and twenty names, of which but sixty-three are those of amateurs and twenty-eight those of foreign dealers and growers. The output of these growers and dealers represents only a portion of the total output of the United States. Almost every florist is more or less interested in the production of corms and flowers of the gladiolus, which appears to be increasing in popularity as a cut flower.

## THE DISEASE

The name *hard rot* was given to the corm stage of the disease under consideration by Wallace (1909:18), who makes no reference to a leaf stage. This name was given to distinguish the disease from other corm

<sup>1</sup> Also presented to the Faculty of the Graduate School of Cornell University, January, 1916, as a major thesis in partial fulfillment of the requirements for the degree of doctor of philosophy.

ACKNOWLEDGMENT. The writer wishes to express his indebtedness to Professors Donald Reddick and H. H. Whetzel, under whose immediate direction the work was conducted, for helpful criticisms and suggestions.

<sup>2</sup> Dates in parenthesis refer to bibliography, page 180.

diseases, such as dry rot and soft rot, with which both Wallace and Fitzpatrick<sup>3</sup> worked prior to 1912. The writer took up the investigation of these diseases in 1912 and has given them constant study since that time.

Apparently the hard rot disease of gladiolus exists wherever gladioli are grown. Specimens have been received from many of the largest growers in the United States. Horticultural publications contain many references to corm rots, and no doubt much of this injury is due to the hard rot disease. Plants growing in the greenhouse at Cornell University from corms received from Italy by A. C. Hottes bore the leaf stage of the disease. The writer has received corms affected with hard rot from Canada, Germany, and Holland.

Prillieux and Delacroix (1894) report having studied a disease of the gladiolus in which the tissue was deeply corroded, but the writer is unable to determine whether or not it is the same disease as the one considered in this bulletin. Unpublished notes placed at the disposal of the writer by Professor F. C. Stewart, of the New York (Geneva) Agricultural Experiment Station, mention the only distinction observed, prior to Wallace's thesis (1909), between two types of rots. Concerning specimens of diseased corms received from a New York State grower, Professor Stewart suggested the probability that they were affected with the bacterial disease described by Prillieux and Delacroix, since he was unable to locate any trace of fungous hyphæ in the diseased tissue. Wallace (1909:15) was of the opinion that the corms received by Professor Stewart were affected with the hard rot disease.

In 1874 Passerini collected specimens of the leaf stage of the hard rot disease, which he contributed to exsiccatae of Rabenhorst's *Fungi Europaei*.

Saccardo (1884) reports the occurrence of the leaf stage of the disease on *Gladiolus segetum* at Parma, Italy, and on *Gladiolus gandavensis* at Coimbra, Portugal. Allescher (1897), in addition to the occurrence on hosts listed by Saccardo, reports the disease as occurring on the leaves of *Gladiolus palustris* in Silesia. So far as known to the writer, the leaf stage of this disease has never been reported in America. A "blight" is frequently mentioned in horticultural publications, but the descriptions of the injury are in all cases so indefinite that it is impossible to determine what diseases the writers had under observation. Hicks (1907:35) and Childs (1907) write of gladiolus leaf blight, but there is nothing in their writings sufficiently definite to make it possible to determine the nature of the injury. Halsted (1894-1901) reports having worked on gladiolus diseases, but leaves the reader in doubt as to what the diseases were.

<sup>3</sup> Unpublished notes of Professor H. M. Fitzpatrick, of Cornell University, covering his investigations of gladiolus diseases, were kindly placed at the disposal of the writer.



Undoubtedly the leaf stage of the hard rot disease occurs more generally throughout the country than is indicated by an examination of literature. This is due to importation of stock from Europe and exchange of stock by growers in this country. On the other hand, the writer has observed specimens of the disease on the foliage of plants grown by but three large growers.

Foliage affected with the disease was first observed by the writer in 1912 in seedling beds, and later on plants grown from cormels. Not until the season of 1915 did the writer find the disease on the foliage of large plants, at which time six plants of flowering size were observed to be affected. In many cases large flowering plants of different varieties have been observed growing in seed beds or among plants from cormels, all of which were badly diseased and yet the large plants showed no signs of the leaf stage. Large plantations of cormels have been observed in which fifty per cent of the plants bore the disease on the foliage.

Nothing has been found in literature that would indicate that there is any relation between the leaf stage and the corm stage of the disease under consideration. It has been the writer's fortune to obtain conclusive evidence that they are but different stages of the same disease, and the experimental data leading to this conclusion are here set forth.

#### ECONOMIC IMPORTANCE OF THE DISEASE

No figures are available to show the economic importance of the hard rot disease of the gladiolus, but it must be considerable as compared with the extent of the industry. Many thousands of corms are discarded during the winter and in the spring at planting time because of their diseased condition. During the summer many thousands of corms fail to reach maturity, due to the decay of the parent corms in the soil. While there are other diseases of the gladiolus, it is the opinion of the writer, based on observations made during the past four years, that a large proportion of this loss is due to the hard rot disease. Several varieties of gladiolus that have been examined showed fifty per cent or more of the corms to be affected by hard rot. So far as the writer knows, no variety is immune.

While the loss caused by the leaf stage of the disease is materially less than that caused by the corm stage, it is still of considerable importance to the grower. It has been observed that when the foliage of seedlings and of plants from cormels is affected by the disease, the corms are smaller than those of plantings that were free from disease. Therefore the decrease in size must be considered along with the total loss of many thousands of corms. To this must be added the extra expense incurred by the grower in sorting and selecting more or less healthy corms from

diseased lots, either in filling orders or for his own planting. All in all, the loss must take from the producer a yearly toll of surprising magnitude.

#### SYMPTOMS

##### *On the leaves*

The first signs of the disease on the leaf are minute brown or purplish brown discolored areas more or less circular in outline. These lesions usually appear in July or early August. The color of the diseased areas deepens somewhat with age until a shade from reddish brown to almost black is reached. Spots of a reddish brown color predominate. In the older lesions there is a well-differentiated center, light gray in color and dotted with numerous black bodies which are very apparent (Plate xv, 1). Surrounding the center is a prominent zone, varying from purplish brown to black in color, which blends into the green of the healthy tissue.

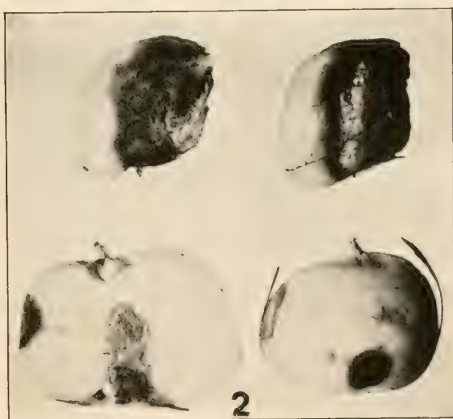
The lesions are more or less circular but with straight sides where they are limited by the midrib and the edge of the leaf. At first the discoloration may be visible on only one side of the leaf, but it soon makes its appearance on the opposite side, so that lesions appear practically identical on either side. They are few or numerous, and vary in size depending on conditions. The coalescence of several or many spots may occur, causing the formation of a single large necrotic area along the entire side of a leaf. Lesions on the tips of the leaves are usually larger and less characteristic than those below. In some cases the ashen gray centers of diseased areas drop out, giving a shot-hole appearance. This is more likely to occur with spots on large flowering plants than with those on seedlings.

##### *On the corms*

Hard rot lesions appear in the fall as minute water-soaked spots, of a reddish brown to brownish black color, usually on the sides and the lower half of the corm but not infrequently on the upper half as well (Plate xvi). It is usually necessary to remove the husks (sheathing leaf bases) from the corms in order to see the lesions, although in some cases the husk also is diseased. The lesion on the husk serves as an indication of the more important lesion underneath. There is no sharp line of demarcation between the healthy and the diseased tissue.

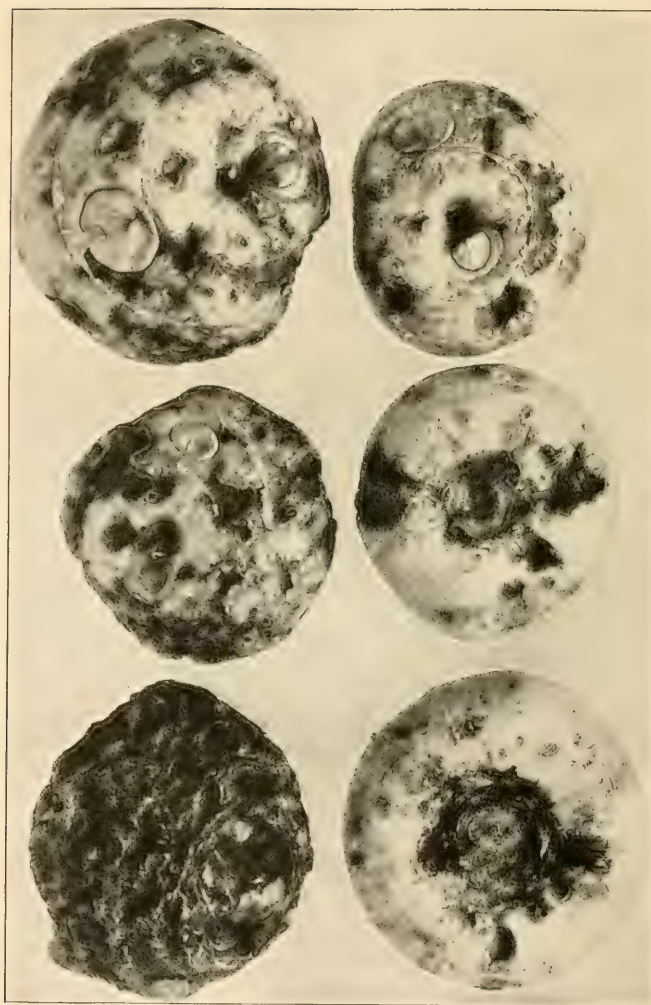
As the spot increases in size, the center becomes sunken, the color deepens to a distinct black, and the margin becomes more definite. A narrow ring, water-soaked in appearance, still indicates the advancing decay. The more definite margin of the older spots is due to the rapidity with which the sunken condition follows the advancing water-soaked area, due to drying of the tissue. The tissue gradually becomes hard, in





## HARD ROT LESIONS ON LEAVES AND CORMS

- 1, Lesions on leaves of gladiolus seedlings.  $\times 2$   
2, Lesions on corms. The two upper corms show lesions well advanced, with the diseased area blending into the healthy tissue. At the bottom the corm on the left has been cut in two in order to show the depth to which the disease has progressed. Natural size



HARD ROT LESIONS ON CORMS

Different stages in the destruction of a corm.  $\times 1\frac{1}{2}$



some cases extremely so, making it difficult to cut the tissue with a sharp knife.

Many small lesions may coalesce into one large lesion, in some cases leaving areas of more or less normal tissue insulated in a large sunken area. Enough tissue not completely decayed may be left to indicate the margins of the formerly separate lesions. Frequently the disease advances so far that the corm is reduced to a hard, shriveled, and wrinkled mummy.

Excepting in very late stages — and in some cases not even then — the lesions do not extend deeply into the corm. The usual range is from one to five or six millimeters (Plate xv, 2). If conditions are not favorable for the development of the rot, the active border disappears, soon assuming the sunken, darkened aspect of the central part. When this stage is reached the diseased tissue can be chipped out with the finger nail, leaving the apparently healthy tissue beneath, as if the disease were not now advancing and the plant had formed a callus over the affected area.

Plants of more or less dwarfed, stunted appearance, which sometimes fail to produce blossoms, are to be found throughout the fields during the growing season. The leaves of these plants usually turn brown and die, the plant having the appearance of having died from drought. In a dry season the number of these plants is unusually large. At this time there is no decay of the new corm which is being developed, but rather the injury is caused by the premature decay of the parent corm before the offspring has developed a sufficient root system to enable it to supply its own moisture and food. This premature decay of the parent corm is not necessarily due to the advancement of the hard rot disease, but probably in most cases to the entrance of saprophytes which cause a rapid disintegration of the corm.

#### ETIOLOGY

The hard rot disease of the gladiolus is caused by the fungous pathogene *Septoria Gladioli* Passer. Passerini collected specimens of the leaf stage of the disease on the foliage of *Gladiolus segetum* near Parma, Italy, in June, 1874, which he contributed to Rabenhorst's *Fungi Europaei* — a collection of exsiccatae material. On this packet of exsiccatae material is written the original description of the fungus.<sup>4</sup> Passerini noted the occurrence of the disease only on the leaves. None of the other investi-

<sup>4</sup> Rabenhorst, *Fungi Europaei*.

1956. *Septoria Gladioli* Passer. hb.

*Perithecia punctiformia atra in macula exarida fulvomarginata: sporae cylindricae subrectae continuae hyalinae cirrose ejectae.*

*Ad folia G. segetum Vigheffio prope Parmam.*

Junio 1874.

leg. G. Passerini.

gators found a sporulating stage of the fungus known to cause the hard rot disease of corms, and consequently the septorial fungus on the leaf was not associated with the organism causing the rot of the corms.

### *Life history*

#### *Pycnidia*

Pycnidia (Plate xv, 1) of the hard rot fungus become visible usually within four or five days after, or in some cases even simultaneously with, the appearance of the lesion on the leaf. They are imbedded in the tissue, but protrude sufficiently to form black papillæ which are visible to the naked eye.

The pycnidia arise from intercellular mycelium (fig. 38). They measure from 100 to 160  $\mu$  in diameter by 60 to 130  $\mu$  high, the

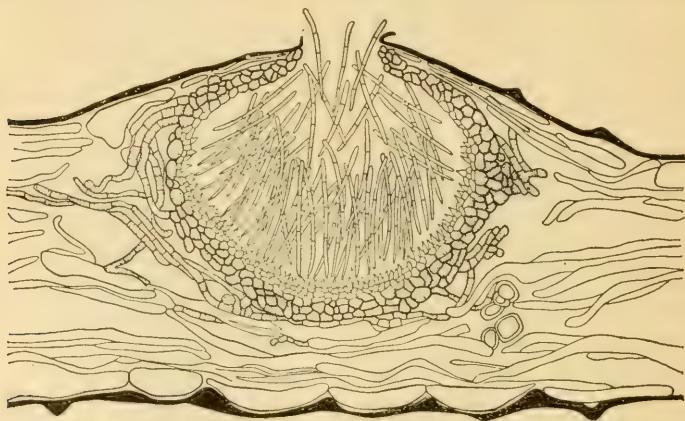


FIG. 38. PYCNIDIUM OF SEPTORIA GLADIOLI

Section through the pycnidium showing how the spores are borne. (Outlined with a camera lucida.)  $\times 333$

average being 127  $\mu$  in diameter by 91  $\mu$  high. The outer wall of the pycnidium consists of pseudoparenchymatous tissue which is brown in color.

From a more or less inconspicuous inner layer of thinner-walled pseudoparenchymatous tissue, hyaline conidiophores arise. From these conidiophores spores are cut off by constriction. In his description of the fungus Allescher (1897) describes the spores as being unicellular and measuring from 30 to 54  $\mu$  long by 2 to 2.5  $\mu$  in diameter. However, an examination made by the writer of specimens contained in packet no. 1956 of Rabenhorst's *Fungi Europæi*, as well as of fresh material, shows that the spores are usually three-septate. As measured by the writer they are from 20 to 55  $\mu$  long by 2.25 to 4  $\mu$  in diameter, the average being about 40 by 3  $\mu$ . The spores from fresh material are cylindrical, almost straight, and hyaline.



When placed in water containing small pieces of leaf tissue, germination occurs in eighteen hours. From one to several germ tubes may develop from a single spore (fig. 39).

### *Mycelium*

The mycelium in the corm is intercellular (figs. 40 and 41). It usually measures from  $1.5$  to  $2.5 \mu$  in diameter, but is in some cases even double this size. The mycelium is septate and varies from olive-brown to black in color.

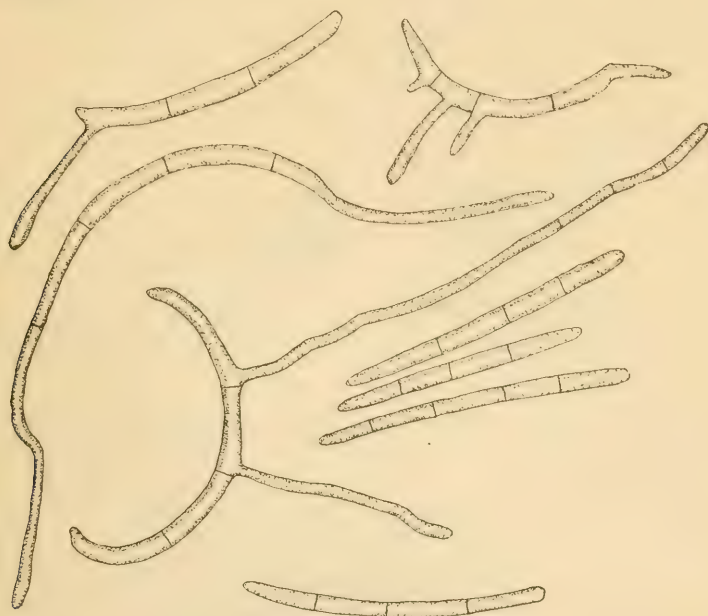


FIG. 39. SPORES OF SEPTORIA GLADIOLI

Some of the spores have germinated. (Outlined with a camera lucida.)  $\times 666$

### *Source of leaf infection*

No sexual stage of the fungus has been found. Old leaves bearing pycnidia when exposed out of doors throughout the winter showed usually only empty pycnidia when examined the following spring. From the results of experiments subsequently discussed, apparently the mycelium of the fungus is able to live over winter in the soil. This suggests the possibility that infection is produced on the foliage by rain splashing soil containing mycelium on to the plants, or by the plants being beaten down on to the soil that harbors the pathogene. However, seedlings around which rye straw was placed to keep them off the ground and to prevent soil from being splashed on to them, were attacked by the fungus

as early and as severely as those not so treated; and attempts to produce infection on the foliage of large plants by bending them over on to the

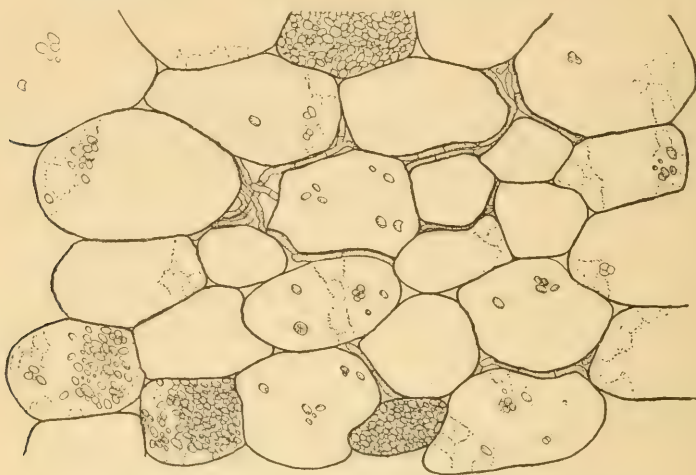


FIG. 40. HISTOLOGICAL EFFECT OF SEPTORIA GLADIOLI

Section of gladiolus corm through diseased tissue. The presence of intercellular mycelium, and the absence of starch in many cells, should be noted. (Compare with figure 41.)  $\times 300$

soil have thus far failed. Not enough work has been done to either prove or disprove these suggested sources of infection of the foliage.



FIG. 41. MYCELIUM OF SEPTORIA GLADIOLI

The intercellular mycelium is shown much magnified. (Camera lucida drawing.)  $\times 600$

In the greenhouse, the incubation period of the fungus on plants that were sprayed with water containing spores in suspension was about twenty days.



*Source of corm infection*

An examination of corms harvested from seed beds where the foliage was badly diseased, has frequently shown from sixty to seventy per cent of them to be affected with hard rot. This, together with the fact that infection was produced on corms by placing in contact with them water containing spores in suspension (page 167), suggests the probability that infection is produced by spores being washed down from pycnidia formed on the foliage to the soil, where they germinate and infect the corms. As seeds are not planted very deeply, this could readily take place. It is unusual, however, for the disease to appear on the foliage of large flowering plants; and as pycnidia have not been observed to be formed on the corm, it seems that the fungus is carried over the winter primarily, if not entirely, in the mycelial stage, no spore form being necessary for the existence of the pathogene.

The fungus can be isolated from lesions on the corms at any time during winter or spring. This shows that the living organism is carried to the soil along with the corm at planting time. The offspring from diseased corms may or may not be diseased. As discussed under control (page 173), selected healthy corms grown in soil in which gladioli have never been grown have without exception given sound offspring. This indicates that the fungus is not a natural inhabitant of the soil. Furthermore, three hundred corms, all of which showed hard rot lesions, were planted in soil in which gladioli had never been grown, and seventy-eight per cent of the offspring bore hard rot lesions. Thus it seems that, in the majority of cases at least, a diseased offspring may be expected from the planting of a diseased corm.

The fungus does not grow directly from the old corm into the new one. This has been determined both by observations and by making numerous cultures from tissue at the juncture of parent and offspring. The fungus must either grow through the sheathing leaf base from the old corm to the new one, or else, as is probably the case, grow out into the soil, from which it attacks the newly developing corm.

No observations have been made which would lead the writer to believe that all infection does not occur in the field. However, it is conceivable that if corms were stored under humid conditions either in contact with one another or with moist soil, the fungus might possibly penetrate a healthy corm from an infected one or from infected soil; or, if they were stored with soil containing the pathogene around them, there is no doubt that, under moist conditions, infection could occur in the storage house as well as in the field.

Diseased corms were minced and placed in soil known to be free from the pathogene, in which two hundred healthy corms were growing. The

pieces of diseased corms were merely sprinkled in among the corms before covering them with soil and no attempt was made to see that pieces were or were not in actual contact with the healthy corms. Seven per cent of the offspring were diseased.

*Longevity of the organism on the foliage and in the soil*

As indicated by the following experiment, the fungus is carried over winter on diseased tops:

Two hundred corms which had been grown for three consecutive years in soil that had never before been used for growing gladioli, were again planted in similar soil in 1915. Previous to planting, the corms were examined and found to be absolutely healthy. After setting the corms, tops from cormels which had been badly affected by the disease the previous year and which had remained out of doors on the ground throughout the winter, were scattered in the row. The tops and the corms were then covered with soil. These plants were harvested in September and the corms stored in a cool place. When examined early in December it was found that eighty per cent of the corms showed hard rot lesions. Practically all the diseased corms had many lesions on them, and the disease was well advanced. *Septoria Gladioli* Passer. was isolated from many of these lesions, proving that this fungus caused the disease. Healthy corms around which no diseased tops were placed but which were otherwise given the same treatment, showed no signs of disease.

The results of experiments indicate that the fungus is able not only to live over winter on old tops on the ground, but also to live in the soil throughout a period of at least four years. In 1915 selected healthy corms were planted in soil in which gladioli had been grown the previous year, and also in soil in which no gladioli had been grown for (a) one year, (b) two years, (c) three years, and (d) four years. During the intervening time the soil had been planted respectively to (a) rye and a crop of rye and vetch, (b) rye and timothy, (c) oats, hay, seeded to clover, cover crop of rye and vetch, (d) grass. In each of the five plots of ground two hundred and fifty healthy corms were planted, two hundred of them being planted in a single row and the remaining fifty in lots of ten at five different places in the field. The corms were harvested in September and stored in a cool place.

Results of these experiments were recorded the following December. Forty-seven per cent of the corms which were grown in the plot in which gladioli had been grown the previous year, were diseased, fifty per cent of the diseased corms showing characteristic hard rot lesions. The corms from the other plots were diseased as follows: (a) twenty-four per cent, forty per cent of which bore hard rot lesions; (b) twenty-three per



cent, thirty-seven per cent of which bore hard rot lesions; (c) fifty-two per cent, eighteen per cent of which bore hard rot lesions; (d) forty-seven per cent, ten per cent of which bore hard rot lesions. Care was taken during the summer to avoid contaminating these plots by introducing affected soil from other fields, and the location was such that it is extremely doubtful that the wind could have entered as a factor. Since healthy corms planted in soil in which no gladioli have been grown give healthy offspring, it follows that the organism must be able to live for at least four years without the presence of the living host. No doubt decaying parts of plants were left in the soil when the last crop was harvested, but it is probable that, at least in the case of plot d, these plant parts were entirely decayed in the four years which intervened between the harvesting of the last crop of gladioli and the planting of the healthy corms used in this experiment.

### *Pathogenicity*

The pathogenicity of *Septoria Gladioli* Passer. was established first for the mycelial stage. The mycelium of the fungus was discovered by Wallace (1909:33), who, after having observed its presence in thin sections of diseased tissue of the corm, succeeded in obtaining the organism in pure culture. He later (1910 a) succeeded in producing the characteristic lesions on experimentally inoculated corms, and reisolated the fungus. Following Wallace, Fitzpatrick (see footnote, page 154) records having produced the characteristic lesions on corms artificially inoculated in moist chambers, from which the fungus was reisolated.

Besides noting the constant association of the mycelium with lesions on corms through microscopical examinations of diseased tissue, the writer has made numerous isolations of the organism from these diseased areas. The growth of the mycelium was studied in pure culture and infection was produced at will, not only in moist chambers in sterile sand, but also in the greenhouse, and in the field under natural conditions.

### *Inoculation experiments*

Corms were selected which after having been in the storehouse for four months showed no signs of disease. This necessitated the removal of the husks. The surface was sterilized by immersing the corm in fifty-per-cent alcohol for three minutes, then in 1-1000 corrosive sublimate solution for ten minutes, and finally rinsing in sterile water. These corms were then planted, some in sterile sand in moist chambers, some in soil in the greenhouse in which gladioli had never been grown, and some out of doors in soil never before used for the growing of gladioli.

For inoculation, mycelium growing in pure cultures on solid media was used. A bit of the medium containing mycelium was removed under sterile conditions, and in some instances smeared over a part of the uninjured surface of the corm; in other cases the corm was first injured by needle punctures and the culture was then smeared on the surface. The corms were permitted to remain in the soil for a period of two or three weeks, when they were removed and the growth they had made was cut off.

In practically all cases one hundred per cent infection was obtained. Most of the corms showed the dark brown, water-soaked areas, characteristic of the hard rot disease, when dug. The remainder showed the lesions very soon afterward. Equally as abundant infection was obtained on the uninjured corms as on those punctured by the needle. From diseased areas of the affected corms the fungus was reisolated and grown in pure culture, where its growth corresponded in every detail with the organism used for the inoculation. Corms similarly treated but having no mycelium placed in contact with them remained healthy in all instances.

In order to further test the ability of the fungus to produce disease, sound corms were planted in soil in which gladioli had never been grown, and permitted to grow to maturity. On August 15, 1914, as the offspring were developing from the parent corms, the soil was inoculated with mycelium of the fungus. The inoculum was prepared by grinding cultures of the organism on oatmeal agar with cornmeal, and was applied by placing a small handful of the mixture around each corm in immediate contact with it. Of the one hundred corms thus inoculated, seventy-three showed characteristic hard rot lesions when the results of the experiment were recorded the following December. Reisolations of the fungus were obtained from many of the diseased corms. Corms from plants which had not been inoculated with mycelium remained absolutely healthy.

The above experiments prove the ability of the mycelial stage of the hard rot fungus to attack gladiolus corms. The experiments given below show that this mycelium is but a stage of *Septoria Gladioli*, which Passerini described as occurring on the foliage of *Gladiolus segetum* in Italy.

A pure culture of the fungus *Septoria Gladioli* Passer. was obtained from the germination of a single spore from a pycnidium formed on the leaf of a gladiolus seedling. The resulting fungous growth was identical with that obtained from isolations from small pieces of diseased corm tissue. Mycelium thus obtained from a single spore was used to inoculate healthy corms, some of which were planted in moist chambers in sterile sand, others in soil in the greenhouse known to be free from the pathogene, and still others out of doors in soil in which gladioli had never been grown. Numerous experiments were performed, and in all cases one hundred



per cent infection was obtained by smearing a small quantity of an agar culture of the mycelium from a single spore on the surface of the corms. The fungus was reisolated from diseased areas on the corms, and its growth in pure culture was found to be identical with the organism previously isolated from corms and from the germination of a single pycnospore.

In order to test the ability of the fungus isolated from a lesion on a corm to attack the foliage, a small piece of an agar culture of the organism was mixed with a little sterile distilled water and painted on the foliage of seedlings and flowering plants growing in the greenhouse. The seedlings were then inclosed in bell glasses lined with moistened filter paper, while the parts of the large plants on which the mycelium was placed were inclosed in a lamp chimney stoppered at both ends with cotton. Seedlings and large plants were similarly treated with mycelium obtained from the germination of a single pycnospore. Plants were similarly treated, except for the omission of the mycelium, to serve as a check.

Inoculations were successful with both the mycelium from the germinated spore and that from the diseased corm. Infection was evident on the seedlings within ten days. The lesions differed somewhat from those found under natural conditions, infection manifesting itself in the form of large, dark, water-soaked areas, with the early death of the entire area over which the inoculum was painted. The lesions produced by mycelium from the two different sources were similar.

On the large plants, infection was observed within fourteen days after inoculation, the lesions being identical on the plants inoculated with mycelium from the two different sources. At first a dark area, water-soaked in appearance, was formed, and then the lesions turned brown due to the death of the tissue. The lesions in no case extended much farther in area than that covered by the culture of mycelium painted on the foliage. The most significant fact is that pycnidia developed in these lesions on the leaf on practically all of the twenty-four plants inoculated with the mycelium, regardless of whether the mycelium was from a germinated spore or from a diseased corm. Although many pycnidia failed to reach maturity, spores were formed in several of them. These spores were germinated and the fungus was obtained in pure culture.

In October, 1914, *Septoria*-like spores were found in a culture of the organism isolated from a diseased corm. These spores, together with others obtained from pycnidia on seedling leaves, were used in the following experiments:

Seeds were planted in three flats in the greenhouse and the plants were permitted to grow until they were from two to four inches high. The plants in one flat were sprayed with water containing, in suspension,

spores from a culture of the fungus isolated from a diseased corm; the plants in the second flat were sprayed with a suspension of spores from pycnidia formed naturally on seedlings; the plants in the remaining flat were sprayed with water containing no spores, for a check. The seedlings were then covered with bell glasses lined with moistened filter paper, and the three flats were placed in a large moist propagating chamber for seventy-two hours.

An examination of these seedlings twenty days after they had been sprayed with the suspension of spores in water showed evidence of infection. Small yellowish brown areas were apparent and numerous pycnidia appeared in these lesions a few days later. The lesions were characteristic of those found on the seedlings under natural conditions. The check plants alone remained healthy, infection occurring on plants which were inoculated either with spores from culture or with spores from pycnidia formed under natural conditions. The fungus was again obtained in pure culture from the germination of single spores from pycnidia formed on both lots of infected plants.

It then seemed desirable to determine whether or not corms could become infected by spraying spores upon them. The surfaces of thirty healthy corms were sterilized by immersing them first in fifty-per-cent alcohol for three minutes, then in 1-1000 corrosive sublimate solution for ten minutes, and finally rinsing in sterile water. Ten of these corms were then planted in each of three moist chambers containing moist sand which had previously been subjected to steam at ten pounds pressure for two hours. Corms that were particularly depressed at the crown were selected for the experiment, in order that a cubic centimeter or more of water could be held in each of these cavities. Water containing spores in suspension was placed in the depressed areas of the corms in two of the moist chambers. The spores for one chamber were obtained from pycnidia formed naturally on the foliage of seedlings, while for the other chamber the spores were obtained from a culture of the fungus isolated from a diseased corm. The third chamber was used for a check, water containing no spores being placed in the cavities of the corms. One-half of the corms in each chamber were then pricked with a sterile needle in the area covered by the water.

Observations made twenty days later showed most of the corms in the two chambers which were inoculated with spores to be infected. Six days later, when they were removed, all the inoculated corms showed the characteristic hard rot lesions, while the check corms remained healthy. Lesions were as abundant on corms that had not been injured as on those punctured with the needle. About one-half of the corms showed hard rot lesions on the sides, where evidently spores had been washed over from



the concave crowns. The fungus was isolated from many of these diseased areas and again obtained in pure culture.

In the spring of 1915 healthy corms were planted in soil in which gladioli had never been grown, and allowed to grow to maturity. On August 21 the soil was removed from around thirty of these plants and water containing a suspension of spores was poured around the corms. At this time the offspring were about one-half inch in diameter. The spores for inoculating fifteen of the corms were obtained from pycnidia formed naturally on the foliage of seedlings, while for the other fifteen corms the spores were obtained from a culture of the fungus isolated from a diseased corm. For a check, corms were given the same treatment except that the water poured around them contained no spores. The soil in which these plants were growing was kept moist for the following three days.

The corms were harvested in the following September and stored in a cool place. When examined in November it was found that ten of the fifteen corms inoculated with spores from pycnidia showed hard rot lesions; also, six of the fifteen corms inoculated with spores from culture showed lesions characteristic of the hard rot disease. The check plants remained healthy. This experiment is significant in showing not only that spores from cultures and from naturally formed pycnidia are able to infect the corms, but also that it is possible for infection to occur on the corms from spores discharged from pycnidia on the leaves. The spores are washed down into the soil, where they germinate and produce infection.

### *Pathological histology*

#### *Leaf*

An examination of thin sections of leaves bearing young diseased areas shows the lesion produced by the fun-

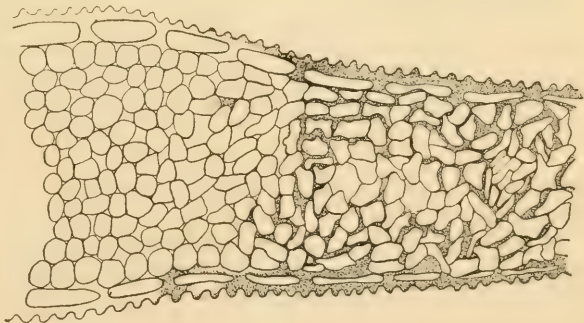


FIG. 42. HISTOLOGICAL EFFECT OF SEPTORIA GLADIOLI

Camera lucida drawing of a free-hand section through a hard rot lesion on the leaf of a seedling. The cells are beginning to shrivel and collapse.  $\times 200$

gus to be necrotic. The cells turn brown, shrivel, and collapse (fig. 42). Here and there a cell may be found filled with a yellow, granular or oil-like substance the identity of which is undetermined. The diseased area is usually but from one-third to one-half the thickness of the healthy tissue.

### Corm

In order to study the histological changes that occur in the corm, comparative studies of healthy and diseased tissues have been made. Both microtome and free-hand sections have been used, the former being less satisfactory because of the difficulty encountered in sectioning prepared material. Sections were stained with a weak solution of iodine in order to study starch content of cells, and with Haidenheim's iron-alum-haematoxylin and aniline blue for a general study of the tissue.

While the cells of healthy tissue are densely packed with starch, those of diseased tissue show but very few starch grains or none at all (figs. 40 and 41 [page 160], and 43). This, together with the deposit in the diseased area of a yellow substance of undetermined composition, is the

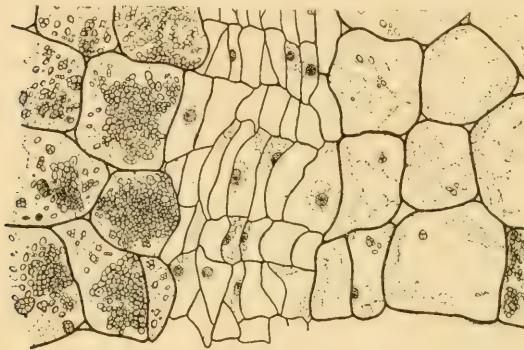


FIG. 43. HISTOLOGICAL EFFECT OF SEPTORIA GLADIOLI

Camera lucida drawing of a microtome section through medium of healthy and diseased tissue. The layer of comparatively thin-walled cork cambial cells separating the starch-filled healthy cells from the diseased cells, which contain little or no starch, should be noted.  $\times 300$

most pronounced effect to be noticed by comparing sections of diseased and healthy tissue. Especially in the early stages of the disease, nuclei and even the cytoplasm appear but slightly disturbed. The cell walls retain their shape for some time after the starch has disappeared. Later, shrinkage takes place and the cells collapse, the walls becoming distorted and broken. This last

effect is no doubt due to loss of moisture rather than to any direct action of the fungus.

A layer of cork cambium is formed at the juncture of the diseased and the healthy tissue (fig. 43). Young, actively advancing lesions do not show this layer of thin-walled cells, but it is to be found in those instances in which it appears that the advance of the disease has been checked and the canker healed. In cases in which the diseased area can be chipped out, the break is at this layer of cork cells.

### Cultural characters of the fungus

Pure cultures of *Septoria Gladioli* Passer. were obtained from isolation plantings of diseased tissue from a corm. The surfaces of corms showing hard rot lesions were disinfected by immersing them in fifty-per-cent alcohol for three minutes, then in 1-1000 corrosive sublimate solution



for ten minutes, and finally rinsing in sterile water. By means of a sterile scalpel the surface of the corm was cut away and a small piece of the tissue at the advancing margin of the lesion was removed to a sterile medium. Comparatively few contaminations were obtained in the large number of isolations made in this manner.

No marked difference was observed in the growth of the mycelium on nutrient or on soil-extract agar, or on other solid media consisting of agar and various plant decoctions, such as of gladiolus, potato, oats, corn, and beans. On the other hand, rolled-oat agar<sup>5</sup> proved slightly more favorable for mycelial growth, and spores were produced by the fungus only when growing on this medium. For this reason rolled-oat agar was used almost entirely for culturing the organism during the last year of study, and the following cultural characters of the fungus are a record of its growth on this medium.

Macroscopically, no growth from bits of diseased tissue placed in medium in previously poured petri dishes is evident

for from seven to fourteen days. However, if the plate is examined under the low power of the microscope, mycelium radiating from the transferred piece of tissue can be seen in about four or five days from the time of making the culture. Frequently the first macroscopical evidence of growth is the appearance of a black growth on the transferred piece of tissue, which may be completely covered before the organism invades the

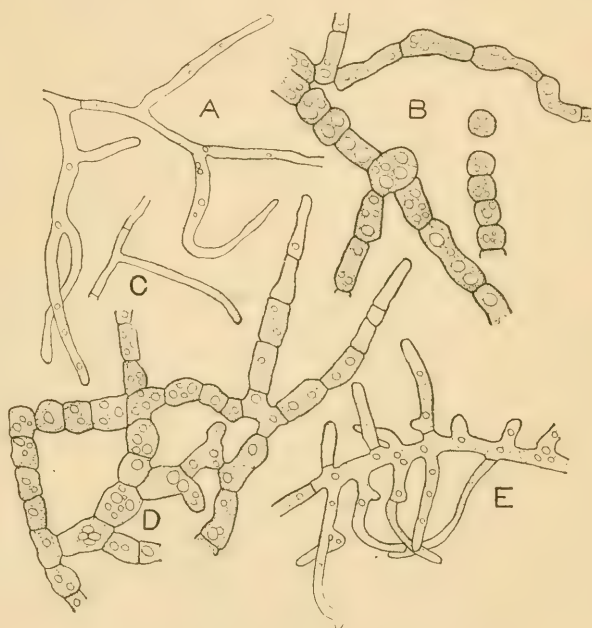


FIG. 44. MYCELIUM OF SEPTORIA GLADIOLI

Camera lucida drawing of mycelium of the hard rot fungus growing on rolled-oat agar. A, colorless strands of hyphae radiating from a bit of diseased tissue. B and D, cell walls that have thickened, the cells having assumed a globose form. C, colorless hyphae to be found in old cultures. E, an intermediate stage between A and B.  $\times 600$

<sup>5</sup>The rolled-oat agar was prepared as follows: 50 grams of rolled oats, in 700 cubic centimeters of distilled water, was cooked in a double boiler for about an hour, or until the oats were thoroughly cooked through. Most of the solid matter was then squeezed through cheesecloth. To this was added 15 grams of agar and enough water to make one liter of medium.

medium. Soon a dense, black colony spreads very slowly into the surrounding medium. After growth of a month the colonies usually do not exceed one or two centimeters in diameter. If portions of a colony are transferred to flasks of media or to other plates, the resulting growth is somewhat more rapid.

Some of the characters of the mycelium as grown in culture are shown in figure 44. The first strands of hyphæ to be seen radiating from the piece of diseased tissue are hyaline (fig. 44, A). Color frequently makes its appearance in streaks, which radiate from the piece of diseased corm, where the hyphæ seem to become gnarled. Cells of the much-septate mycelium thicken, assuming a globose form (fig. 44, B). Well-defined globular bodies, which appear to be oil drops, soon appear within the cells. Osmic acid causes these to turn brown. The walls turn brown with the appearance of these bodies, giving the colony its black color when viewed macroscopically. The globose or subglobose cells of the hyphæ may remain attached, forming chains, or may separate into individual cells (fig. 44, B).

Although the growth is usually confined beneath the surface of the medium, small scant patches of white, aerial mycelium are found occasionally. The hyphæ of old cultures is of two kinds: one of comparatively long, colorless cells measuring from 1.5 to about  $4\mu$  in diameter (fig. 44, C, E); and one of short, thick, globose cells containing the oil drops mentioned above, measuring from 3 to 6 or  $7\mu$ , or sometimes even  $12\mu$ , in diameter (fig. 44, B, D).

Scattered through the colonies are areas which under the microscope appear denser and blacker than other areas. These seem to be caused by a gnarling, or balling, of the hyphæ at these points, together with the anastomosing of cells of different hyphæ, as if pycnidia or other fruit bodies were to be formed. Cultures have been examined intermittently throughout a period of over three years, and no further development of these masses of hyphæ has been observed.

Spores of *Septoria Gladioli* Passer. were first observed in culture in October, 1914. The mycelium on which these spores were formed was isolated in the preceding June, from a hard rot lesion on a corm. This mycelium was allowed to grow in a tube of rolled-oat agar from June until August 20, when a square block of the medium containing mycelium was transferred to the slanted surface of about 200 centimeters of rolled-oat agar contained in a 300-cubic-centimeter Erlenmeyer flask. The medium contained in this flask was freshly prepared. There were about 10 cubic centimeters of water of condensation in the flask at the base of the slanted medium. The culture from which the transfer was made was well dried at the time when the square of medium containing mycelium

was removed, and this condition may have influenced spore formation when the mycelium was placed on the freshly prepared medium.

By approximating the above conditions the writer has been able to bring about the formation of spores in cultures of the mycelium from other sources than the one above noted. Spores were formed in a culture of the mycelium obtained from the germination of a single pycnosporer formed naturally on the leaf. Spores formed in cultures of mycelium isolated from corms and from germinated pycnosporers were identical in shape and size, thus materially helping to establish the identity of the two previously unconnected organisms.

Spores in culture have always appeared as minute pinkish white pustules on the upper edge of the block of medium containing mycelium transferred from the old culture. Later these pustules may appear scattered over the surface of the medium of the flask to which the transfer was made. If at this time transfers are made from this flask to another, the pustules are formed more readily and in greater abundance.

Owing to difficulties encountered in obtaining sections or mounts of these pinkish white elevations, but little is known of their structure, especially in reference to the formation of the spores. Normal pycnidia such as those formed on the foliage are not produced. The spores are formed in a very loose stromatic mass. There is an abundance of dense, pinkish white mycelium, which is still in evidence after spores are no longer to be found associated with the pustules.

Spores formed in culture are variable in size, ranging from 25 to 97  $\mu$  by 1.8 to 3.75  $\mu$ , the average being 58 by 2.71  $\mu$ . Dilution plates of these spores were made in nutrient agar and practically one hundred per cent germination was obtained within a period of eighteen hours. The resulting mycelial growth was not so brown in color as that isolated from corms. Many minute, black dots appeared, which, when examined under the microscope, proved to be aggregations of short, thick-walled cells formed commonly and more abundantly in cultures of the fungus isolated from corms. Transfers were made from these plates to tubes of rolled-oat agar, where the resulting growth was identical with that obtained by isolations made from diseased corms.

In order to correlate the growth of mycelium isolated from diseased tissue of corms with that isolated from the leaf, dilution plates of spores from naturally formed pycnidia were made. From these dilution plates individual spores, which were so located that they could be removed singly, were transferred to other plates where germination was observed under the microscope. The resulting mycelial growth in all cases has been identical with that isolated from corms when the two were growing under similar conditions.



## CONTROL

The great need of some method of combating the organisms causing rots of gladiolus corms was early impressed upon the writer, and many suggested methods of general application were tried for the control of the rots collectively rather than separately. Another disease, designated by Wallace (1909:61) as dry rot, was found to be present along with the hard rot disease in stock which was used in all control experiments. The lesions produced by the fungi causing these two diseases are so similar that they can be distinguished only in the earliest stages, and not even then with a great degree of accuracy. Cultural isolations of the organisms will often show a lesion to have been caused by the hard rot fungus when it was selected as being a dry rot lesion, or vice versa. Not only are the lesions produced by the two fungi similar, but the life histories of the organisms are not materially unlike except for the fact that no spore form of the dry rot fungus has been found. From all indications, a treatment applicable to the control of one disease should be of value in controlling the other. At least fifty per cent of the corms used for experimental purposes were affected with the hard rot disease. This estimate is based on observations and cultural studies throughout a period of several years. In practically all cases, after the corms were treated, the organisms have been isolated from diseased areas in order to make it absolutely certain that both were present, and in no case has any treatment resulted, so far as the writer was able to judge, in materially changing the ratio of the corms affected by the two diseases.

In view of the fact that control experiments were conducted previous to, and simultaneously with, life history studies, it is not surprising that some treatments which at first seemed worthy of trial failed to bring results. Many of the following treatments have given negative results. This does not wholly deprive them of their value, for they serve to narrow down the field of experimental possibilities of control. Many data have been obtained from the treatments which will be valuable in a further study of control measures.

## SEEDLING TREATMENTS

The hard rot disease on the foliage of seedlings has been materially reduced by spraying with bordeaux mixture used at the strength of five pounds of copper sulfate and five pounds of lime to fifty gallons of water. In 1914 the first spray was applied on July 17. This application was followed by eight other treatments made at intervals of about seven days. Because of the smooth surface of the foliage, it was necessary to use a "sticker," or adhesive, to cause the fungicide to adhere to the plants. The "sticker" used consisted of resin two pounds, sal soda (crystals)

one pound, and water one gallon, which, after being boiled until a clear brown color was obtained, was added to each fifty gallons of the bordeaux solution. The seedling beds were sprayed twice the same day for each application, the second spray being applied as soon as the first was dried on the foliage. This was done in order to thoroughly cover the plants. A hand sprayer was used, in which a pressure of from three and one-half to five pounds could be maintained at all times.

H. H. Groff, of Simcoe, Ontario, informed the writer that he was successful in controlling a disease of the foliage of seedlings by spraying with a solution of copper sulfate in water. Specimens of the disease sent by Mr. Groff to the writer proved to be the hard rot disease. From the nature of the foliage of the gladiolus, it is probable that the plant is more resistant to spray injury than are most plants and that a solution of copper sulfate could be used without causing injury. However, no experiments have been conducted by the writer using copper sulfate solution as a spray for the control of this disease, and it is very unlikely that results could be obtained, because the copper sulfate would be washed away with the first rain.

Although spraying will greatly reduce the amount of disease on the foliage, a simpler and more efficient method is to plant the seed in soil in which gladioli have never been grown. When this was done, and care was taken not to carry parts of diseased plants or soil bearing the fungus to these seedlings, it was found that not a single diseased plant appeared during the summer. The corms of these plants were materially larger when harvested than the corms of plants whose foliage was attacked by the hard rot fungus, and no evidences of disease on the corms were observed. This is the logical way to control the hard rot disease, which causes so much damage in seedling beds. It is doubtful whether any grower plants such a large quantity of seed, or has such a limited area of ground, that soil in which gladioli have never been grown cannot be obtained for this purpose. If this plot is kept isolated and care is taken not to introduce the pathogene into the soil, there appears to be no reason why seedlings cannot be grown on the same area year after year, if necessary, at least so far as the hard rot disease is concerned.

#### CORM TREATMENTS

##### *Healthy corms in soil free from the pathogens*

Selected healthy corms have been grown for the past four years in soil in which no gladioli had ever before been planted, without a single corm's becoming diseased. The fact that these corms were stored throughout each winter in a room containing diseased corms leads to the con-

clusion that the fungi causing the hard rot and dry rot diseases are not disseminated in storage. It is obvious that in order to obtain results from the selection of healthy corms, rigid and painstaking care must be exercised to select corms known to be absolutely free from disease. Any doubtfully healthy corms must be rejected, for a single diseased corm may serve to infect the soil in which healthy corms are planted.

To select healthy corms it is necessary to remove the husks and to be sure there is no evidence of disease on the corms. It is best to do the selecting in the spring, as near planting time as possible, for, whereas a corm may be infected in the fall at digging time and still show no evidence of being diseased, the lesion is sure to be noticeable by planting time. Previously to planting these corms it is advisable to treat them with a five-per-cent solution of formalin for thirty minutes, in order to kill any parts of the pathogenes which may be clinging to them.

In 1912 from two thousand to three thousand healthy corms were selected in the manner suggested above. They were planted each year in soil that had not been under cultivation for about twenty years. A commercial phosphate fertilizer was applied to the experimental plots at the rate of about five hundred pounds to the acre, and the corms were planted in the usual manner. Care was exercised to see that no foreign soil nor diseased plant parts were introduced into these plots. The plants received the usual amount of cultivation and were subjected to the same conditions as commercially grown plants. Spikes of flowers were cut during the blooming season, and the corms were harvested and stored each autumn in the ordinary way.

This process of selecting healthy corms and growing them in soil free of the pathogenes is the only means known that will give an absolutely healthy crop. Of course the large amount of labor, the carelessness of laborers, the need of a larger outlay of land, and the inability to procure land on which gladioli have never been grown or at least not for many years, are some of the important factors that will at once suggest themselves to growers, especially the larger growers who produce many thousands of corms annually. It is admitted that this is a slow and somewhat undesirable method from many standpoints, yet it is a process that has proved conducive to results, and undoubtedly can find some application by all growers. Small growers can readily and with no great loss adopt such a method for growing gladioli. Larger growers can adopt the process in part.

Such a method could be begun on a small scale, by selecting as many healthy corms the first year as conveniently possible and planting them in soil in which gladioli had never been grown. More selected corms could be added to this lot the second year, and so on until the grower



gradually worked away from diseased to healthy stock. The opportunity for healthy corms to become diseased is thus lessened, and diseased conditions are in general improved.

*Healthy corms in soil known to harbor the pathogenes*

When selected healthy corms were planted in soil in which gladioli had been grown the previous year, the offspring were diseased. The amount of disease varied from twenty-three to forty-seven per cent. The possibility suggested itself that some treatment might be devised which would protect the offspring of the sound corms that were planted, from the pathogenes that must be in the soil.

An experiment was conducted in 1914 in which the corms of the various plots were treated with different chemicals. A small handful of the chemicals was placed over each corm previously to covering the corms with soil. The chemicals used were: plot 1, sulfur; plot 2, air-slaked lime; plot 3, acid phosphate; plot 4, soot. The soot was suggested by a grower who claimed to have obtained good results through a liberal application of this substance to the soil. The plants received ordinary cultivation during the summer, and the offspring were harvested and stored in the usual manner. In December, when the results of the experiments were recorded, it was found that none of the treatments were of any value, the percentages of disease in the treated plots being practically the same as that in a check plot where no treatment was given. The experiment was repeated in 1915 with the same results.

*Diseased corms in soil free from the pathogenes*

*Spring treatments*

When diseased corms that had received no treatment were planted in soil free from the pathogenes, it was found that the offspring gave various percentages of disease. Seventy-eight per cent of the offspring from three hundred corms bearing typical hard rot lesions, which were planted in soil free from the pathogenes, were diseased. In other instances, thirty-three and fifty-seven per cent diseased offspring, respectively, were recorded from the planting of two lots of three hundred corms affected with either hard rot or dry rot, or both.

An experiment was conducted in 1914 to determine whether or not some treatment could be given these corms at planting time which would lessen the amount of disease in the offspring when the corms were grown in soil free from the pathogenes. Corms that bore typical hard rot lesions, and others that were affected with either the hard or the dry rot disease or both, received the following treatments: (1) formalin at the rate of

one pint of commercial formalin to fifteen gallons of water, for eighteen hours; (2) corrosive sublimate, 1-1000 solution, for eighteen hours; (3) chemicals, in which the corms were rolled and with which they were covered after being placed in the rows and before covering them with soil. The chemicals used were sulfur, air-slaked lime, acid phosphate, and soot. The corms were planted in soil in which gladioli had never before been grown, and received ordinary cultivation during the summer.

When the corms were examined in December, 1914, the results obtained indicated that none of the treatments were effective in reducing the amount of disease. The experiment was repeated in 1915, with the same results except that corms over which a handful of sulfur was placed were injured severely by the chemical. Such treatments of diseased corms have proved to be of no value in controlling the hard rot and dry rot diseases.

#### *Autumn treatments*

Since the lesions on corms attacked by the hard rot and dry rot organisms are materially smaller in the autumn when the corms are dug than in the winter, it was thought that possibly the corms could be given some treatment at digging time whereby the pathogenes within the tissues would be killed. Consequently the following experiments were performed with the hope of at least lessening the extent of injury to the corms.

*Experiment 1. Treatment of corms with formalin and corrosive sublimate solutions.*—In 1914 one thousand corms, of which many had lesions in various stages of advancement at digging time, were treated, immediately after digging, with formalin at the strength of one pint of commercial formalin to fifteen gallons of water, in which they were left for eighteen hours. An equal number of corms were treated with 1-1000 corrosive sublimate solution for eighteen hours, and an equal number were left untreated for a check. After treatment the corms were cured out of doors and then stored as usual.

In the following December, when the results of these treatments were recorded, it was found that neither had reduced the amount or the extent of the diseases. Thirty-five per cent of the offspring from the untreated corms were diseased, while thirty-seven and thirty-eight per cent, respectively, of the offspring from the corms treated with formalin and corrosive sublimate solution were diseased.

The same experiment had been performed the previous season (1913), with the result that about ninety per cent of the corms of both the treated lots were diseased while the corms in the check were but seventy per cent diseased. This remarkable situation is difficult to explain. There was a prolonged period of wet weather about the time the treatments

were made, so that the corms remained wet for about a week after they were treated. The corms were either injured by being subjected to the action of the reagents for so long a time, or else the increased percentage of diseased corms was due to some other abnormal condition brought about by the wet condition of the corms. Many of the corms bore lesions which were not characteristic of either the hard rot or the dry rot disease, from which neither the dry rot organism nor *Septoria Gladioli* Passer. could be isolated.

*Experiment 2. Formaldehyde gas as a disinfectant.*—On the basis of successful experiments performed for the control of potato scab by the use of formaldehyde gas, diseased corms were subjected in 1913 to a similar treatment. Obviously this would eliminate the humid condition arising from the use of solutions.

In this experiment the gas was generated by the potassium permanganate method. At harvesting time one thousand corms were placed, immediately after digging, in shallow trays in a large air-tight box. The formaldehyde gas was obtained by using enough potassium permanganate to generate gas at the rate of three pints of formalin and twenty-three ounces of permanganate crystals to five hundred cubic feet of space, it having been previously determined that corms thus treated were unharmed. The treatment extended over a period of twenty-four hours. The corms were then thoroughly cured in the open air and stored as usual.

In January, 1914, when the results of this treatment were recorded, it was found that sixty-nine per cent of the corms were diseased while seventy per cent of the untreated corms from the same lot were diseased. The hard rot and dry rot organisms were isolated from many lesions, showing both organisms to be alive. The difference of one per cent in the amount of disease can easily be explained on the basis of experimental error, with the resulting conclusion that formaldehyde gas as used in this experiment is of no value in controlling the corm rots of gladioli.

*Experiment 3. Hot-water and hot-air treatments of diseased corms.*—In a third experiment some means was sought whereby diseased corms could be subjected to heat, which would kill the organisms within the tissue without causing injury to the corms. After such treatment the corms could be planted in soil known to be free of the pathogenes and be depended on to yield a healthy crop.

Previously to conducting the experiments it was determined that the thermal death point of the hard rot and dry rot organisms was about 50° C. when subjected to this temperature in a test tube culture for a period of ten minutes. The tubes were immersed in the hot water as soon as new growth appeared from pieces of medium containing mycelium which were transferred to the tubes. It was also previously determined



that corms of from three-fourths inch to one and one-half inches in diameter, when subjected to dry heat at 50° C. for one and one-half hours or to water at this temperature for one-half hour, were not materially harmed.

Having thus obtained some idea of the relative resistance of both corms and the two pathogenes to heat, corms were subjected in 1913 to dry heat and to water at 50° C. for one and one-half hours and one-half hour, respectively, and the progress of the disease was noted. There was enough difference between the length of time required to kill the fungi and that which caused no injury to the corms to warrant this treatment. The corms used were of a single variety and showed considerable disease when dug. They were treated on the same day that they were harvested. For the hot-water treatment a half-bushel galvanized iron measure was used, the heat being supplied by an oil-stove flame, and for the dry-air heating a Freas electric oven was used. After treatment the corms were cured as quickly as possible and then stored in a cool place as usual. Wet weather lengthened the time necessary to thoroughly cure the corms more than was desirable.

In the following January, when the results of this experiment were recorded, it was found that seventy per cent of the untreated corms bore lesions of either the hard rot or the dry rot disease, while of those treated with dry heat and hot water eighty-five and ninety-five per cent, respectively, were diseased. In both cases in which treatments were given, the corms, besides containing a large percentage of disease, showed the lesions to be more advanced than those in the check. Both the hard rot and dry rot organisms were isolated from many diseased corms of both lots, showing the pathogenes to be still alive.

In accounting for the increased percentages of disease in the treated over the untreated corms, it was found that many of the lesions, besides being different in appearance from those produced by the two fungi, were identical with those produced on healthy corms which were subjected to heat under the same conditions and from which neither the hard rot nor the dry rot organism could be isolated. These lesions were undoubtedly due to injury caused by the heat. However, a sufficiently large number of corms bore characteristic lesions of the two diseases, and the causal organisms were isolated from enough lesions, to prove that the treatments were a failure in killing the fungi within the tissue at a temperature that would not injure the corm.

#### SOIL TREATMENTS

*Experiment 1. Chemicals.*—Since the organisms causing the hard rot and dry rot diseases are able to live over winter in the soil, the possibility

suggested itself that some chemical might be applied to the soil which, either through its toxicity or by its ability to change the composition of the soil thereby rendering it unsuited for the existence of the pathogens, would serve to eradicate them. Healthy corms could then be planted safely in this soil.

For the experiment in 1912 a plot of land was used on which gladioli had been grown for the past three years. The chemicals used and the amounts per acre were as follows: air-slaked lime, 1200 pounds; sulfur, 1000 pounds; air-slaked lime 800 pounds, and sulfur 1000 pounds; sulfate of iron, 1800 pounds; acid phosphate, 1200 pounds; acid phosphate, 2100 pounds. The chemicals were applied by the use of a lime spreader, in strips of 10 by 136 feet, a strip of equal width being left between each of the treated areas to serve as a check. The entire experiment was conducted in triplicate. Across the strips and at right angles to them were planted the rows of corms, each row consisting of a single variety. During the growing season special care was taken to see that no soil was carried from one treated area into another or into the checks. The results of the treatments were based on corms removed from a center seven-foot strip of each of the treated and the check areas.

In the following January, when the results of this experiment were recorded, it was found that none of the treatments had been of any value. No reduction whatever was obtained in the amount of disease in treated as compared with untreated corms. Since the chemicals were applied in as large amounts as is commercially practicable, if not larger, no further soil treatments with chemicals have been tried on a large scale.

*Experiment 2. Formalin as a soil disinfectant.*—Soil in which seedlings had been grown for the past two years was treated in 1912 with one gallon of one-per-cent formalin solution per square foot. The plot was covered with heavy burlap for two days after being treated. As soon as the odor of formaldehyde could no longer be detected, seeds were planted in the treated soil, other seeds being planted in untreated soil to serve as a check.

During the summer the hard rot disease appeared on the foliage of these seedlings. No doubt infection occurred from spores blown from diseased seedlings growing near by in untreated soil. The corms were harvested in the autumn and stored in a cool room.

In the following January, when the corms were examined, it was found that seventeen per cent of those grown in treated soil were diseased, while thirty-seven per cent of the corms from untreated soil showed disease. Since the disease appeared on the foliage of these plants during the summer, it was impossible to determine whether the source of infection was the mycelium of the fungus in the soil, or spores that might have been washed

down from the leaves to the soil where they would germinate and infect the corms. Therefore it was impossible to determine from this experiment whether or not the formalin treatment had been of value as a soil disinfectant. The experiment was repeated in 1915, but no results were obtained because the seed planted failed to germinate.

*Experiment 3. Formalin as a soil disinfectant.*—The value of formalin as a soil disinfectant for *Septoria Gladioli* Passer. and the dry rot fungus was further tested by treating soil in which gladioli had been grown for the past two years with formalin at the rate of one gallon of one-per-cent solution per square foot. Healthy corms were planted in this soil. No lesions of the hard rot disease appeared on the foliage during the summer. In the following January, when the corms were examined, it was found that the treatment had proved of no value in reducing the percentage of disease, as compared with healthy corms growing in untreated soil. However, the treated plat was not sufficiently isolated from other untreated areas to preclude the possibility that infected soil might have been carried from untreated soil to that which was treated, and hence the results must be considered with that restriction.

#### SANITATION

It has been shown that the hard rot fungus is able to live over winter on dead tops left lying about on the ground. It follows that these tops should be raked up in the fall and burned. This suggestion applies particularly to the tops of seedlings and cormels, since the disease has been observed by the writer to occur on the foliage of but six plants of flowering size. It has also been indicated that the fungi causing the hard rot and dry rot diseases of gladioli will live in the soil for at least four years. Care should therefore be taken that the soil does not become infected with the pathogenes. Only healthy corms should be planted in soil which it is desired to keep free from these fungi; at least more care should be exercised at planting time to see that no corms badly diseased are planted. Such corms should be discarded and burned, for they will but decay in the soil and infect it with the disease-producing organisms. Crop rotation should be practiced.

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